

Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 1 of 11

1/11

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K at I	T 39 tta Y	E 90 atgg G	C gcca H	gctg W acaa N	gta Y itgo A 45	aac T	I aac T	D 410 aaa K	C gga E	Q Q aga E	iaaa K iact L	D aac T 470	A tac T	E 4 cat M	I 30 gat I	a
K at I	T 39 tta Y	E 90 atgg G	C JCC: H	yctg W acaa N	gta Y itgo A 45	I aac T 50	I :aac T	D 410 aaa K	gga E	cca Q aga E	K K nact L	D aac T 470	A tac T	E 4 cat M	I 30 gat I	a
K at I	T 39 tta Y	E 90 atgg G	C JCC: H	gctg W acaa N	gta Y itgo A 45	aac T	I aac T	D 410 aaa K	gga E	cca Q aga E	K K nact L	D aac T 470	A tac T	E 4 cat M	I 30 gat I	a
K at I ga E	T 39 tta Y agc	E 90 atgg G gtgg	C geca H gaga E	gctg W acaa N aatg	y Y A 45 1998	I T SO atga E	iaac T ngct	D 410 aaa K ctt L	gga E geg R 51	Q aga E E ICC <u>Q</u> R	iaaa K iact L stgt	D aac T 470 aaa K	tac T	E 4 cat M aaa	I 30 gat I	a g
K at I ga	T 39 tta Y age R	E 90 atgg G gtgg G	C geca H gaga E 490 ttt	gctg W acaa N aatg	y X X X X X X X X X X X X X X X X X X X	I T SO atga E	aac T igct L	D 410 caaa K ctt L	gga E geg R 51	aga E CCS R	aaaa K act L ytgt V	D aac T 470 aaa K	tac T ggt V	E 4 cat M aaa K	I 30 gat I gcc P	a g
K at I ga E	T 39 tta Y age R	E 90 atgg G gtgg G	C geca H gaga E	gctg W acaa N aatg	y X X X X X X X X X X X X X X X X X X X	I T SO atga E	aac T igct L	D 410 caaa K ctt L	gga E geg R 51	aga E CCS R	aaaa K act L ytgt V	D aac T 470 aaa K	tac T ggt V	E 4 cat M aaa	I 30 gat I	a g
K at I gg G G 30	T 39 24 39 39 39 39 30 30 30 30 30 30 30 30 30 30 30 30 30	E 90 atgg gtgg G att	C geca H gaga E 490 ttt	gctg W acaa N aatg W	y tgc A 45 ggg a D	I T SO atga E	I aac T igct L caac	D 410 aaa K ctt L	gga E geg R 51 gtac	aga E ICCS R .0	aaaa K L stgt V	D aac T 470 aaa K	A tac T ggt V gat I	E 4 cat M aaa K	I 30 gat I gcc P	a g 5
K at I gs E gs G G 30 gs	T 39 49 R 99 D 0 gaa	E 90 atgg gtgg G att	C gcca H gaga E 490 ttt F	gctg W acaa N aatg	y A 45 D Atgr	I aac T 60 at ge	I aac T agct L aac	D 410 caaa K cctt L gcgg	gga E gcg R 51 gtac	aga E CCG R O	kaaa Kact Latgt V	D .aac T .470 .aaa K atgo	A tac T ggt V	E 4 cat M aaa K	I 30 gat I gcc P	a g 5

FIG._1A



Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 2 of 11

2/11

590 610 ttatatgattatgaccgaaaagatgcagaaggcaagctgcgcgagctt Y D Y D R K D A E K L R E G 650 630 catctgaaaaagagcattgaagtgatagaggtcccgtctattccagaa LKKSIE VIEVPSI 690 710 cggcatacagttcaccatgaacaaattgaggatttgcttacaacgaca TVHHEQIEDLLTT 750 730 ttgattgaatgcgcttacttttcggtggggaaatggaacttatcagga C A Y F S V G K W N LIE 70 790 tcagcaagcttaaagcagcaaaaaccattccttcttatcagtgtgatt ASLKQQKPFLLISVI 830 850 gaaggggagggccgtatgatctctggtgagtatgtctatcctttcaaa S G E Y V Y P F K GEGRM I 890 aaaggagatcatatgttgctgccttacggtcttggagaatttaaactc G D H M L L P Y G L G F.K.L E 930 gaaggatatgcagaatgtatcgtctcccatctg C·I A E

FIG._1B



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180	NEYSE	••	KGFTL	09	240 AAKTD	: : G-KTE	; 	290 ↓↓ NKVDHA	# : . :	1			нтунн	230
г	IRTGNE		NGMY		REKGPY	: : : : : EYANIHENGELG-KTE	110	280 290 ↓ -GGIFVGPCGNKVDHA	<u></u>	YVPSGT	170	VKN	PSIPER	0
170	IEGIIK	••	SOSVVQ	20	230 2RYCRS1	: -EYANI	;	280 GGIF	•• ;	KPGDFF	160	340 TSSFYP	IEVIEV	220
-	carpa.p vindgdvnipervdwrokgavipvknogscascwafsavviiegiikirigninerse	·· 	PLFFKPVFKERIWGGTALADFGYTIPSQRTGEÇWAFAAHQNGQSVVQNGMYKGFTL		190 200 210 220 240 _carpa.p lldcdrrsygcnggypwsalqlvaqygihyrntypyegvqrycrsrekgpyaaktd	:::: VHPND	100	LYR	_	OCQKDAEIIYGHNATTKEELTTMIERGEWDELLRRVKVKPGDFFYVPSGT	Ä	300 310 320 330 340 AVGYGPNYILIKNSWGTGWGENGYIRIKRGTGNSYGVCGLYTSSFYPVKN	: AIGKGILALETQQNSDTTYRLYDYDRKDAEGKLRELHLKKSIE VIEVPSIPERHTVHH	210
160	GSCWAI	= = -	rgeçwa]	40	22 IHYRNT	:: :: : :: ::: :: :: :: :: :: :: :	:	260 SIANQPVSVVLEAAGKDFQLYR-	<u>-</u>	GEWDEL.	150	330 GTGNSY	EGKLRE	
150	VKNQGS	· .:	ripsor'	30	210 LVAQYG	: LDADQD	90	270 SVVLEAA	••	TTMIER		320 GYIRIKR	YDRKDA	200
r-i	KGAVTP		LADFGY	<i>:</i>	PWSALO	 	0	260 IANQPV	••	TTKEEL	140	3 GWGENG	TYRLYD	0
140	<i>(</i> VDWRQ)		[WGGTA]	20	200 NGGY]	l : LEGDRF	. 80	 	-	IYGHNA	130	¥ 310 KNSWGT	ZONSDT	190
	OVNIPE		VFKER		RSYGCI	: : \HLFGQ]	70	250 PYNEGAL	**)KDAEI:	ਜ	PNYILI	ILALET(180
130	VLNDGI		PLFFKE	10	190 LLDCDE	LWEHHE		250 papa_carpa.p RQVQPYNEGALLY	••	YIIDC(120	300 AVGYG]	: AIGKG	
ĵ.	pa.p	:			pa.p	;		pa.p		· ·		carpa.p	ete si	
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Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 4 of 11

4/11

							, ,					1. 1.			•
	IGMYKG	=	IGPYKG		. ← .	SKTECW	=	SKTECW	ਜ [ਾ]	1	/ HAIGKG		HALCKG	्त =	
20	IGOSVVQN	- - -	GPSTVAN	20	110	HENGELO	= ::	NEEGELG	110	170	VPSGTV	=======================================	VPSGTL!	170	٠.
40	MITEPLFFKPVFKERIWGGTALAD-FGYTIPSQRTGECWAFAAHQNGQSVVQNGMYKG		ITQSPIFLTPVFKEKIWGGTALRDRFGYSIPSESTGECWAISAHPKGPSTVANGPYKG	T ₄₀	100	SELWEHHRHLFGQLEGDRFPLLTKILDADQDLSVQVHPNDEYANIHENGELGKTECW		IELWEEHREVFGGVEGDRFPLLTKLLDVKEDTSIKVHPDDYYAGENEEGELGKTECW	100	160	DCQKDAEIIYGHNATTKEELTTMIERGEWDELLRRVKVKPGDFFYVPSGTVHAIGKG		DCKENAEIIYGHTARSKTELVTMINSGDWEGLLRRIKIKPGDFYYVPSGTLHALCKG	160	`.
30	-FGYTIPSQRT	- :: :: :: :: :: :: :: :: :: :: :: :: ::	REGYSIPSEST	30	06	KILDADQDLSV	: :::::::::::::::::::::::::::::::::::::	KLLDVKEDTSI	06	150	IERGEWDELLR	= ::	INSGDWEGLLR	150	
20	RIWGGTALAD-		KIWGGTALRDF	20	08	LEGDRFPLLTR		VEGDRFPLLT	80	140	ATTKEELTTMI	<u>-</u>	ARSKTELVTMI	140	i
10	PLFFKPVFKE	:::	PIFLTPVFKE	10	70	WEHHRHLFGQ:	= ::	WEEHREVFGG	. 07	130 T	KDAEIIYGHN	::	ENAEIIYGHT	130 T	5.
	MTTE	=	MTOS		09	LSEL	=	LIEL	:	120	IDCC	=	IDCK		
(59 yjde.pep		PMI	KT 60		19 yjde.pep	I,	PMI	ž 1. 20	; 0	yjde.pep	- -	PMI	T	0.8

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Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 5 of 11

5 / 11

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T. Carlo	+ TRADENS

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	♦↓ ALETQQNSDTTYRLYDYDRKDAEGKLRELHLKKSIEVIEVPSIPERHTVHHEQIEDLL	: : : : : : : : :			TLIECAYFSVGKWNLSGSASLKQQKPFLLISVIEGEGRMISGEYVYPFKKGDHMLLPY		TFVQGEYFSVYKWDINGEAEMAQDESFLICSVIEGSGLLKYEDKTCPLKKGDHFILPA		·	
230	нео	l: Tesi		0	В	Ξ	GDH			*
(4)	TVH	DES	230	290	FKK	-	LKK	290	:	* .
	ERH	GYI		.'	VYP	••	CTCE	2		
220	SIE	: PHVD			SGE	••	YEDI		:	t.
8	EVE	 ATVE	220	280	RMI	••	LLK	280		÷ .
	IEV.	VNA	••		GEG		GSG	;	:	•
210	KKS	H:		·	VIE	=	VIE			
	THT	. – : J.HE	210	270	TIE	<u></u>	SICS	270		\$
	KLRE	: II	(4		KPFI	-	ESFI		÷	:
ō	AEGI	:: l SNG		,	KQQ	•••	AQD		:	· .
200	RKD	 RLD	200	260	ASL		AEM	260		
	QXQ.	 DYD	7	·	SSS		INGE			코프보
0	RLY	I:I	.* .*		KWNI	<u>::</u>	KWD			LIVSH
190	TTX	: 	↓ 190	250	SVG	_	SVYI	250	310	YAEC
=	→ ISNO	ISNO	← Ä	,	AYE	=	EYF	7		GEFKLEGYAECIVSHL : :: : PDFTIKGTCTLIVSHI
.*	ETQ.	 ETQ			IEC	••	VQG			EFKL :-: DFTI
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	deb			٠	dec		:		5	d e d
	de r	· H			99 yjde.pep		н	:		yjde.pep PMI
	39 yjde.pep	TT PMI	Д Д О	•	9 7	TS	PMI	E 8	•	PMI PMI
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-1G._3B



Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 6 of 11

6/11

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	MYKG	-	PLAG		H	TECW	=	TECW	; · · · · :	ਜ ਜ	IGKG	<u>-</u>	LCKG	·. ·.
20	ZNGOSVVQNG	<u>:</u> ::	AHGSSSVKNG	50	110	NIHENGELGK		KLHENGDLGR	110	170	FYVPSGTVH		FYVPSGTLH	170 TI
40	GECWAF AAH(GECWAVSAH	T 40	100	QVHPNDEYA		QVHPDDDYA	100	160	RVKVKPGDF	= ::	RIKIKPGDF	160
30	FGYTIPSQRT	=======================================	FGYAIPSQKT	30	06	ILDADQDLSV		LLDANMDLSV	06	150	ERGEWDELLR		ESGDWNGLLR	150
20	MTTEPLFFKPVFKERIWGGTALAD-FGYTIPSQRTGECWAFAAHQNGQSVVQNGMYKG		MTHPLFLEPVFKERLWGGTKLRDAFGYAIPSQKTGECWAVSAHAHGSSSVKNGPLAG	20	. 80	LSELWEHHRHLFGQLEGDRFPLLTKILDADQDLSVQVHPNDEYANIHENGELGKTECW	-:	LDQVWKDHPEIFGFPDGKVFPLLVKLLDANMDLSVQVHPDDDYAKLHENGDLGKTECW	: 08	140	▼▼ IDCQKDAEIIYGHNATTKEELTTMIERGEWDELLRRVKVKPGDFFYVPSGTVHAIGKG		IDCKDDAELILGHHASTKEEFKQRIESGDWNGLLRRIKIKPGDFFYVPSGTLHALCKG	140
10	FFKPVFKER.	=======================================	FLEPVFKER	10	70	ннкнгесог	- :: - :: ::	COHPEIFGFP	. 02	130)AEIIYGHNA'		рептення	130
	MTTEPI	=	MTHPI		09	LSELWE	•••	LDQVWK	09	120	IDCQK	=======================================	IDCKD	120
	59 yjde.pep	FT	YDHS	KŢ		19 yjde.pep	Į,	YDHS	I,	ć (/y yjde.pep	् प्	YDHS	1

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Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 7 of 11

7/11

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8	EDLL	: Gnae		7	ILLPY	-	TILPA	·		; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ;
230	зкнтуннеоі	: : :: KVHTPEVKEV	230	290	VYPFKKGDHM		QYECNAGSHE	290	:	:
220	EVIEVPSIPI	(: AEVITIPHID	220	280	SEGRMISGEY		SSGRIINNGI	280	:	:
210	Lrelhlkks	:: : KRTLHIEKAN	210	270	(PFLLISVIE)	<u>:</u> =)TYLLGSVLS	270		
200	YDRKDAEGK	: : : YDRCNDQGQ	200	260	GSASLKQQK	••	GRAAFPSYC	260		2.
190	♦♦ LETQQNSDTTYRLYDYDRKDAEGKLRELHLKKSIEVIEVPSIPERHTVHHEQIEDLL		11	250	TLIECAYFSVGKWNLSGSASLKQQKPFLLISVIEGEGRMISGEYVYPFKKGDHMLLPY		YVQSDYFSVYKWKISGRAAFPSYQTYLLGSVLSGSGRIINNGIQYECNAGSHFILPA	250	0 310 GEFKLEGYAECIVSHL	EFTIEGTCEFMISHP
180	ALETQ	: VLEIQ	180	240	TLIEC	••	SÕAĀA	240	300 GEFKI	GEFTI 300
	39 yjde.pep	TT YDHS	II		99 yjde.pep	T 5	; YDHS	HF.	yjde.pep	YDHS

FIG._4B



Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 8 of 11

8/11

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50							70	+++	+ ~ ~	cta	cac		9 206		205	
gga	G	gac T	gaa K	gct1 L	R	D.	A A	F	G	Y	A	I	P	S	Q	ıa
				110			-		•	1	30					
aaa	aac	agg	tga	gtg	ctg	ggc	cgt	ttc	tgc	aca	tgc	cca	tgg			g
K	T	G	E	C	W	A	V	S	A	Н	Α	Н	G	s ·	S	
	15							170		٠					90	
tct S				tgg: G				agg G					tca Q	'		g
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				gct. L						gga D	L	S	cgt V	gca Q	agt V	c
90						3	10			-			33	0		
cat	tcc P	tga D	_	tga D						.cga E			cga D		tgg G	jτ
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aaa	aac	gga	gtg	gctg	gta	tat	cat	tga	ttg			itga	cgc	cga	act	a
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G	D	F	F	Y	V	P	S	G	T	٠	н	A	T	С	K	
30							550				_ 4		57			
gg			_	tcct L				agca Q		icto S		tac T	aac T	ata Y	tcg R	1C
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FIG._5A



Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 9 of 11

9/11

610 590 gtatacgattatgaccgctgtaatgaccagggccaaaaaagaactctt V Y D Y D R C N D Q G Q K R T 630 650 catatagaaaaagccatggaagtcataacgataccgcatatcgataaa IEKAMEVITIPHIDK 690 710 gtgcatacaccggaagtaaaagaagttggtaacgctgagatcattgtt V H T P E V K E V G N A E I I V 750 730 tatgtgcaatcagattatttctcagtgtacaaatggaagattagcggc YVOSDYFSVYKWK I S G 790 70 cgagctgcttttccttcatatcaaacctatttgctggggagtgttctg RAAFPSYQTYLLGSVL 830 850 agcggatcaggacgaatcataaataatggtattcagtatgaatgcaat SGSGRI N NGIQY I 870 890 gcaggctcacactttattctgcctgcgcattttggagaatttacaata I L P A H F G E G S H F 930 gaaggaacatgtgaattcatgatatctcatcct F M I S H CE

FIG._5B



Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 10 of 11

10/11

			10						3						*
ato	aco	gcaa	atc	acco	gat	ttt	tct	aac	gcc	tgt	gtt	taa	aga	aaa	aatc
M	T .	Q	S	P	I	F	L	T	P	V	F	K	E	K	I :
50			•				70				:		9		
										tgg	ata	cag			ttca
W	G	G	T	A	L	Ŗ	D	R	F	G	Y	S	I	P	s
				110					.		30				
															accg
E	S	T	G	E	C	W	A	Τ.	5	A	н	P	K	G	P
	15							170							90
ago	cac	tgt	tgc										gat	cga	gctt
S	T	V	A	N	G	P	Y	K,	G	K	T	L	I	E	L
					21	0			•			230			·
tgg	gga	aga	gca	ccgi	tga	agt	att	cgg	cgg	cgt	aga	ggg	gga	tcg	gttt
W	E	E	Н	R	E	V	F	G	G	V	E	G	D	R	F
			50						27					:	2
CC	gct											tac	gtc	aat	taaa
P	L	L	T	K	L	L	D	V	K	E	D	T	S	I	K
90			-				10	•				:	33		
gti														aga	acto
V	H	P	D	D .	Y	Y	Α	G	E	N	E	E	G	E	L
				350							70				ţ
			gga	atg	ctg	gta	cat	tat	cga	ctg	taa	gga	aaa		agaa
G	K	T	E	С	W	Y	I	I	D	.C	K	E:	N	A	E
	39							410					•		30
at	cat	tta	cgg	gca	tac	ggc	CCG	icto	aaa	aac	cga	act -	tgt	cac	aatg
I	I	Y	G	Н	T	A	R	S	K	T	E	L			М
					45							470)	# 3- i	
at	caa	cag	cgg	gtga	cto	gga	ggg	rcct	gct	gcg	aag	aat	caa	aat	taaa
I	N	S	G	D	W	E	G	L	L	R	R	I	K	I	K
		. 4	90						51	.0					5
															gtgc
P	G	D	F	Y	Y	V	P	S	G	T	L	Н	A	L	С
30							50			•			57		
aa	aaa	ggc	cct	ttgt	ttt	aga	gac	tca	igca	aaa	itto	aga	tgc		atac
K	G	Α	L	V	L	E	\mathbf{T}	Q	Q	N	S	Ŋ	Α	${f T}$	Y

FIG._6A



Bacillus Subtillis with an Inactivated Cysteine Protease-1 David A. Estell SN# 09/462,846 Docket No. GC381-US Sheet 11 of 11

11 / 11

590 610 cgggtgtacgattatgaccgtcttgatagcaacggaagtccgagagag R V Y D Y D R L D S N G S PR 630 650 cttcattttgccaaagcggtcaatgccgccacggttccccatgtggac AVNAATVPH H F A K 690 710 gggtatatagatgaatcgacagaatcaagaaaaggaataaccattaaa GYIDESTESRKGITIK 750 730 acatttgtccaaggggaatatttttcggtttataaatgggacatcaat T F V Q G E Y F S V Y K W D I 70 790 ggcgaagctgaaatggctcaggatgaatcctttctgatttgcagcgtg G E A E M A Q D E S F L I C S V 830 850 atagaaggaagcggtttgctcaagtatgaggacaaaacatgtccgctc KYEDK EGSGLL 890 870 aaaaaaggtgatcactttattttgccggctcaaatgcccgattttacg K G D H FILPAQMPD 930 ataaaaggaacttgtacccttatcgtgtctcatatt I K G T C T L I V S H I

FIG._6B